

# A new approach to integrate toxicity grade and repeated treatment cycles in the analysis and reporting of phase I dose-finding trials

A. Doussau<sup>1,2,3,4,5\*</sup>, R. Thiébaut<sup>3,4,5,6</sup>, B. Geoerger<sup>7,8</sup>, P. Schöffski<sup>9,10</sup>, A. Floquet<sup>11</sup>, M. C. Le Deley<sup>12</sup>, S. Mathoulin-Pélissier<sup>5,11</sup>, E. Rizzo<sup>13</sup>, P. Fumoleau<sup>14</sup>, C. Le Tourneau<sup>2,15</sup> & X. Paoletti<sup>1,2</sup>

<sup>1</sup>Department of Biostatistics, Institut Curie, Paris; <sup>2</sup>U900, INSERM, Paris; <sup>3</sup>CIC1401-Clinical Epidemiology, INSERM U897, Bordeaux; <sup>4</sup>Division of Public Health, University Hospital, Bordeaux; <sup>5</sup>CIC1401-Clinical Epidemiology, Bordeaux University, Bordeaux; <sup>6</sup>Labex Vaccine Research Institute, Bordeaux; <sup>7</sup>Pediatric and Adolescent Oncology, Institut Gustave Roussy, Villejuif; <sup>8</sup>CNRS UMR8203, University Paris-Sud 11, Villejuif, France; <sup>9</sup>Department of General Medical Oncology, University Hospitals Leuven, Leuven Cancer Institute, Leuven; <sup>10</sup>Laboratory of Experimental Oncology, Department of Oncology, KU Leuven, Leuven, Belgium; <sup>11</sup>CIC1401-Clinical Epidemiology, Institut Bergonié, Bordeaux; <sup>12</sup>Biostatistics and Epidemiology Unit, Institut Gustave Roussy, University Paris-Sud 11, Villejuif, France; <sup>13</sup>EORTC-Headquarter, Brussels, Belgium; <sup>14</sup>Comprehensive Cancer Center, Centre Georges-François Leclerc, Dijon; <sup>15</sup>Department of Medical Oncology, Institut Curie, Paris and Saint-Cloud, France

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**Background:** Safety assessment beyond the dose-limiting toxicity evaluation period provides relevant information to define the recommended phase II dose (RP2D) of a new treatment. We retrospectively analyzed three phase I trials to illustrate two indicators: per-cycle probability of graded toxicity and cumulative probability of severe toxicity over the treatment period.

**Patients and methods:** Data were collected from two continual reassessment method (CRM) trials (T1: aviscumine in solid tumors with short time on treatment; T2: erlotinib + radiotherapy in brainstem gliomas with longer time on treatment) and one 3 + 3 design (T3: liposomal doxorubicin + cyclophosphamide combination in ovarian carcinoma). The probability of severe and moderate or severe toxicity per cycle was estimated at each dose level with mixed proportional odds model. The cumulative probability of severe toxicity was also estimated with the time-to-event CRM.

**Results:** Eighty-three patients were included in the three trials; 94, 96 and 72 treatment cycles were administered, in T1, T2 and T3, respectively. Moderate toxicities were at least twice as frequent as severe toxicities. An increased probability of toxicity over time was detected in T3 [ $P = 0.04$ ; per-cycle probability of severe toxicity: 27% (cycle 1) to 59% (cycle 6) at the RP2D]. At the RP2D, 37% of patients experienced at least one severe toxicity over the first six cycles in T2, and 78% in T3.

**Conclusions:** Dedicated methods can be used to analyze toxicities from all cycles of treatment. They do not delay accrual and should be integrated in the analysis and reporting of phase I dose-finding trials.

**Key words:** dose-finding trials, phase I, dose-limiting toxicity, longitudinal studies, cumulative toxicity, statistical analysis

## introduction

Phase I oncology clinical trials are designed to evaluate the toxicity profile of a new treatment and to identify a dose that can be safely recommended for phase II trials (RP2D). Classically, the RP2D is a dose associated with a predefined probability of severe toxicity (grade 3, 4 or 5 according to the NCI CTC-AE scale), often between 20% and 30%. The main end point, called dose-limiting toxicity (DLT), is usually evaluated during the first cycle of treatment. This period of observation is also sometimes called

‘DLT window’. Two classes of methods have been developed to find this dose, sometimes called algorithmic designs, such as the traditional 3 + 3 design, and model-based designs, such as continual reassessment method (CRM). Regardless of the statistical method used, a considerable volume of the collected information is not formally analyzed to identify the RP2D, as only severe toxicities collected in the DLT window are taken into account for dose finding.

Phase I trials provide much more information than the sole evaluation of DLT, including the graded severity of toxicities, as well as repeated measurements of toxicities throughout the treatment period. In a retrospective study of 445 patients included in phase I trials of molecularly targeted agents, more than 50% of severe toxicities occurred after the first cycle of

\*Correspondence to: Dr Adélaïde Doussau, Department of Biostatistics, Institut Curie, 26, rue d'Ulm, 75248 Paris Cedex 05, France. Tel: +33 1-56-24-56-47; E-mail: adelaide.doussau@isped.u-bordeaux2.fr

treatment. The occurrence of cumulative or late toxicities is also feared, not only due to previous examples such as cardiac toxicity of anthracyclines, but also because some agents are delivered over prolonged period of time. The DLT-TARGETT initiative led by the European Organization for Research and Treatment of Cancer (EORTC) recommended taking into account certain lower grades of toxicities and toxicities that occur after the first cycle in assessment of the RP2D [1].

However, simply extending the DLT evaluation window raises, many issues including delayed accrual and nonassessable patients in case of early drop out for progressive disease. Furthermore, selection bias due to dropouts cannot be excluded in such series, as the majority of phase I trial participants remain on study only for a short period of time.

Some methodological alternatives are available. The objective of this study was to illustrate how the enriched information of the toxicities experienced through the whole treatment period can be rigorously analyzed to refine the RP2D, without delaying patient accrual. Repeated measurements of toxicities have rarely been used in this context. An extension of the CRM was recently proposed to either prospectively use repeated assessments of graded toxicity or to retrospectively reanalyze the collected data to refine the estimate of the RP2D and to investigate modification of the probability of toxicity over time [2]. These approaches provide investigators with a probability of toxicity at each cycle. If this probability increases with time, then cumulative or late effects cannot be excluded.

Another approach, Time-To-Event CRM (TITE-CRM), provides estimates of the cumulative probability of toxicity over several cycles. It was proposed to address the issue of late toxicities in radiotherapy dose-finding trials [3]. This method is not based on repeated events, but on the first severe toxicity and it cannot be used to investigate time trends.

To illustrate clinically how toxicity occurring after the first cycle of treatment can be integrated in the final definition of the RP2D, we retrospectively analyzed three completed phase I clinical trials of anticancer agents corresponding to two situations: two agents administered for numerous cycles of treatment and one agent administered for few cycles. We compared the results obtained from two analyses based on: (i) modeling of repeated measurements of graded toxicity to identify a possible increased probability with successive cycles and to estimate the per-cycle probability of moderate or severe toxicity at each dose, and (ii) estimation of the cumulative probability of severe toxicity over the treatment period. We show the added value of these approaches compared with using only events occurring during the traditional DLT window.

## methods

### characteristics of the three trials

General characteristics of the three trials and toxicity profiles are summarized in Supplementary Tables S1 and S2, available at *Annals of Oncology* online.

**Aviscumine trial.** The EORTC carried out a CRM phase I trial of i.v. aviscumine, an *Escherichia coli*-derived recombinant type II ribosome-inactivating protein, in patients with solid tumors [4]. Forty-one patients were evaluated at 14 increasing doses ranging from 10 to 6400 ng/kg. Four DLTs were observed: one grade 3 fatigue (4000 ng/kg) and three grade 3 liver

toxicities (at 4800 and 6400 ng/kg). At the end of the trial, the RP2D was 5600 ng/kg (13th dose).

**ERLOTINIB trial.** The European consortium Innovative Therapies for Children with Cancer (ITCC) carried out a CRM phase I trial of erlotinib, a tyrosine kinase inhibitor of the Epithelial Growth Factor Receptor, in combination with radiotherapy in children with pontine glioma [5]. Twenty children were evaluated at three increasing doses of erlotinib ranging from 75 to 125 mg/m<sup>2</sup>. Two DLTs were observed: fatal grade 5 seizures, grade 3 skin rash and pruritus (at the first and last dose levels, respectively). The third level was recommended.

**Caelyx®-cyclophosphamide trial.** A 3 + 3 phase I dose-finding trial of the combination of pegylated liposomal doxorubicin and cyclophosphamide was conducted in patients with early recurrence of ovarian carcinoma [6]. Twenty-one patients were evaluated at five dose levels ranging from 35 to 45 mg/m<sup>2</sup> for doxorubicin and 500 to 600 mg/m<sup>2</sup> for cyclophosphamide. DLTs were observed in two patients at the fifth level: grade 3 esophagitis with grade 4 neutropenia and grade 3 thrombocytopenia; grade 3 stomatitis and vomiting with constipation and asthenia. The fourth level was recommended (doxorubicin 40 mg/m<sup>2</sup> + cyclophosphamide 600 mg/m<sup>2</sup>).

### data collected

We focused on the first six cycles of treatment and used the cycle as time scale. All adverse events at least possibly related to treatment were collected.

To evaluate the per-cycle probabilities of severe or moderate toxicities, the outcome of interest was the worst grade of toxicity at each cycle, recoded as: 1 = no or grade 1 (mild) toxicity, 2 = moderate toxicity (grade 2) or 3 = severe toxicity (grade 3–5). In all trials, the definition of DLT then approximately corresponded to the 'severe' toxicity outcome; severe toxicities could be observed at all cycles.

### statistical analysis

Each of the three trials was reanalyzed separately to provide: (i) per-cycle probabilities of severe and moderate or severe toxicity, (ii) cumulative probability of severe toxicity over six cycles.

**per-cycle probability of severe toxicity, and moderate or severe toxicity.** At each dose and each cycle, a percentage of patients experience outcomes. The probability of severe toxicity and the probability of moderate or severe toxicity can then be estimated. Both probabilities are assumed to increase with the dose and correspond to the dose-toxicity relationships. In addition, it can be assumed that increasing the dose has the same effect on the probability of severe toxicity and the probability of moderate or severe toxicity. The proportional odds model is a natural extension of the logistic model when the outcome is an ordered categorical variable [7]. A mixed-effect proportional odds model (POMM) was used to take into account the correlation between repeated observations for a given patient [2]. This model is also an efficient mean to account for missing at random data. Indeed, patients stop treatment due to severe toxicity or progression, and outcomes that would have occurred after a patient dropped out can be seen as missing data. Under the assumption that (i) the risk of progression at a dose is largely independent on the risk of toxicity, (ii) the risk of going off study for toxicity is only related to the dose and to previously observed outcomes, maximum likelihood estimates of mixed models provides unbiased estimates.

Let  $Y_{ij}$  be the outcome that can take values  $k = 1$  (mild), 2 (moderate) or 3 (severe) for patient  $i$  at cycle  $j$ . Let  $d_i$  be the dose allocated to the patient when (s)he is enrolled in the trial.

$$\log \left( \frac{P(Y_{ij} \leq k | d_i, t_{ij})}{1 - P(Y_{ij} \leq k | d_i, t_{ij})} \right) = \alpha_k - \beta_1 d_i - \beta_2 t_{ij} - u_i \text{ with } u_i \sim N(0, \sigma_0^2)$$

Note that  $\beta_2$  estimates the effect of time. No interaction between dose and time is modeled due to the lack of power to detect interaction with phase I sample sizes. Details are provided in supplementary Appendix A, available at *Annals of Oncology* online.

**cumulative probability of severe toxicity.** We also investigated the probability for a patient to experience a severe toxicity during the first six cycles of treatment, as in the TITE-CRM [3]. The relationship between the dose and the time to first severe toxicity was modeled by a weighted logistic function (see details in supplementary Appendix A, available at *Annals of Oncology* online). In this approach, observations of patients without severe toxicity are down-weighted proportionally to the length of follow-up; for instance a patient who dropped out after three cycles and who did not experience severe toxicity was attributed a weight of 0.5. The cumulative probability after six cycles was also represented using the Kaplan–Meier (KM) method.

In the trial of a combination of two molecules (Caelyx®-cyclophosphamide, CACY), the dose of each molecule was included separately, by assuming no interaction between the two molecules. In all models, 95% confidence intervals (CIs) of the estimated probabilities were calculated by a multivariate delta method. We computed a measure of the relative gain in precision of the final estimate of the probability of severe toxicity: precision was quantified as half the length of the 95% CI and gain was the relative improvement in precision using repeated ordinal data over the precision using binary data at the first cycle only.

## results

The distributions of outcomes are presented for each trial in terms of doses and cycles in Figure 1. The estimates for the POMM are given in supplementary Table S3, available at *Annals of Oncology* online.

### aviscumine trial: a trial with short time on treatment

At the end of the trial, the estimated probability of DLT at cycle 1 was 16% (95% CI 7% to 37%) at the RP2D. Of the 97 cycles administered to the 41 patients included, 94 cycles delivered up until the sixth cycle were selected (mean: 2.3 cycles/patient, Table 1). Grade 2 and 3 toxicity were the worst grades in 34 and 7 cycles respectively, including 17 grade 2 and 2 grade 3 that occurred after the first cycle. Seven patients experienced at least one severe toxicity over the six cycles.

The probability of toxicity did not vary significantly with time ( $P=0.27$ ). As shown in Table 1, the estimated probability of severe toxicity at each cycle at the RP2D was 13.6% (95% CI 5.8% to 27.6%), which means that, for each cycle received, the probability of experiencing severe toxicity was 13.6%. The probability of moderate or severe toxicity was 60.6% (95% CI 42.7% to 76.3%). The relative gain of precision obtained by analyzing ordinal repeated data was 27% compared with the CRM on cycle 1 and binary data only.

TITE-CRM analysis was not relevant in this trial, as all grade 3 toxicities occurred during the first two cycles.

### ERLOTINIB trial: a trial with longer time on treatment

The probability of DLT at the RP2D was 16% (95% CI 4% to 45%) according to the CRM [5]. A total of 96 cycles were delivered up to the sixth cycle in 20 children (mean: 4.8 cycles/child,

Table 1). Nineteen and 7 cycles with worst grade 2 or grade 3–5 toxicity were recorded respectively, including 4 cycles with severe and 13 cycles with moderate toxicity outcome experienced after the DLT window. Six patients experienced severe toxicities over the six cycles.

The probability of toxicity did not vary over time ( $P=0.83$ ), suggesting the absence of cumulative effect. The estimated per-cycle probability of severe toxicity was 8.6% (95% CI 3.7% to 18.7%) at the highest investigated dose (Table 1). The width of the 95% CI was strongly reduced by 63% when compared with analysis restricted on the DLT window only. The estimated probability of moderate or severe toxicity per cycle was 30.8% (95% CI 18.7% to 46.3%).

Using the TITE-CRM approach, the probability for a child to experience severe toxicity over the first six cycles was 37.2% (95% CI 12.2% to 71.5%) at the RP2D. This estimation was very close to the estimation obtained with the KM approach (supplementary Figure S1, available at *Annals of Oncology* online).

### CACY trial: an increased probability of toxicity with increasing cycles

Twenty-one patients received 77 treatment cycles, including 72 cycles delivered up until the sixth cycle (mean 3.4 cycles/patient, Table 1). The worst grade experienced at each cycle was 33 grade 2 and 18 grade 3 or 4; 9 grade 3 or 4 and 14 grade 2 toxicities occurred after the DLT window. Eleven patients experienced severe toxicities over the six cycles.

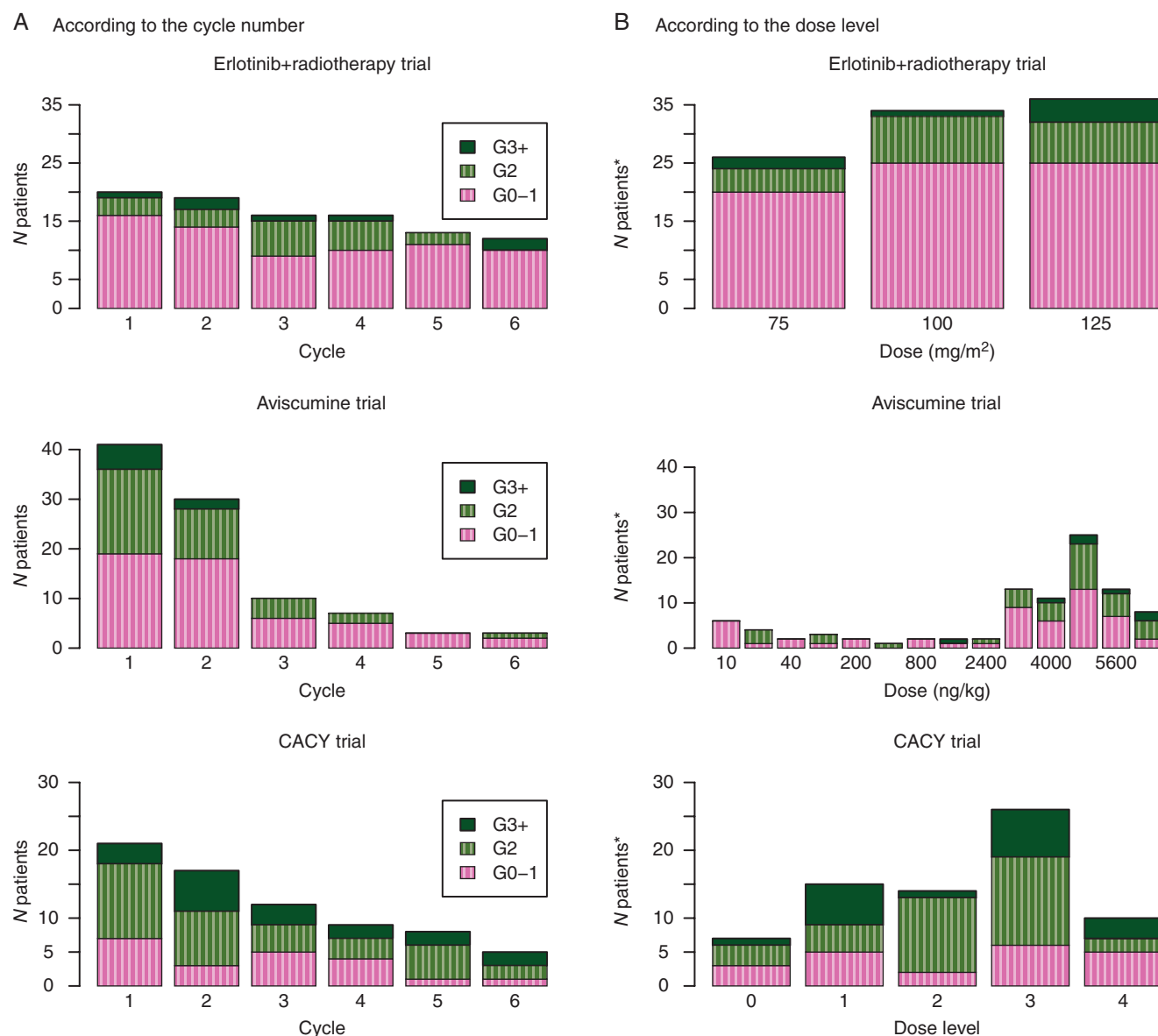
A significant time trend ( $\beta_2 = 0.42$ , odds ratio (OR) = 1.52 per treatment cycle,  $P=0.04$ ) was detected. The estimated probability of severe toxicity at the RP2D increased from 27.1% (95% CI 8.8% to 56.3%) at cycle 1 to 59.2% (95% CI 25.6% to 86.7%) at cycle 6 (supplementary Figure S2, available at *Annals of Oncology* online); the probability of moderate or severe toxicity was 72.4% at the first cycle (95% CI 44.1% to 90.5%) and 92.2% at the sixth cycle (95% CI 66.4% to 99.9%). The gain in precision could not be evaluated, as no confidence interval was estimated in the 3 + 3 trial.

In the TITE-CRM analysis, the cumulative probability of severe toxicity over six cycles was 78.2% (95% CI 41.4% to 94.8%) for the RP2D. The estimate was 67.7% (95% CI 28.5% to 85.4%) using the KM approach (supplementary Figure S1, available at *Annals of Oncology* online).

## discussion

This retrospective analysis of three completed clinical trials illustrates the additional information provided by global modeling of the severity of safety data collected at all treatment cycles in three situations (short and longer follow-up and increased probability of toxicity with time). Moderate or severe toxicities can be analyzed to refine estimation of the safety of the RP2D; more than one-half of toxicities were experienced after the first cycle. A previously unreported time effect was detected for one trial.

Two approaches were distinguished. The POMM analysis provides estimates of the probabilities of severe and moderate toxicity per cycle. The number of observations used was markedly increased by 2-, 3- or 5-fold (in the aviscumine, CACY and erlotinib trials, respectively), compared with analysis based on a



**Figure 1.** Distribution of the frequency of worst grade of toxicity experienced during the cycle, over the number of observations per cycle (A, left part) and dose level (B, right part). \*Total superior to the number of patients allocated to each dose, as each patient could contribute to several cycles. In the aviscumine trial, the severity of laboratory toxicities that had worsened from baseline was recorded. Lymphocytopenia was coded as 'moderate' or 'mild' when the grade worsened by 2 or 1 point(s) from baseline, respectively; for other laboratory toxicities, grade 3 and 2 toxicities were coded as 'moderate' or 'mild' if they had worsened by 1 point from baseline. In CACY trial, dose levels corresponded to the combination of doses of doxorubicin and cyclophosphamide, as reported in Table 1.

single observation per patient, resulting in narrower confidence intervals around probability estimates. With three or six patients at each dose level, crude toxicity rates may be misleading and may differ from the model estimates that incorporate the constraints of an increasing logistic relationship. In addition, moderate toxicity, provided additional information to refine the RP2D estimates and to detect a time effect. The good operating characteristics of this method have been investigated in the statistical literature [2]; however, this is the first illustration of the recent POMM approach on real phase I trials. Alternatively, the time-to-event approach allowed estimation of the probability for a patient to experience at least one severe toxicity during follow-up. These methods are more applicable and useful for the

erlotinib and CACY trials than for the aviscumine trial, where most patients dropped out after cycle 2.

We did not consider dose modifications here; while in our trials, only five patients had dose reduction, the DLT-TARGETT reported that relative dose intensity was below 80% on average. Modeling the administered dose may then be required. We also assumed that the probability of toxicity complied with the proportional odds assumption, which implies that the effects of the dose as well as of the time are the same for severe toxicities and for moderate or severe toxicities. It is likely that the same mechanism is responsible for severe and moderate toxicities, which makes the proportional odds assumption reasonable. Would this hypothesis not seem reasonable, then more flexible models,

**Table 1.** Observed and estimated probabilities of toxicity according to the dose

Aviscumine trial <sup>a</sup>	Aviscumine dose (ng/kg)					Total
	3200	4000	4800	5600	45/500	
Number of patients	4	6	10	7	5	41
Number of cycles	13	11	25	13	8	94
Number of moderate toxicities	4	4	10	5	4	34
Number of severe toxicities	0	1	2	1	2	7
Observed moderate or severe toxicity (per cycle) %	30.8	45.5	48.0	46.2	75.0	43.6
Observed severe toxicity (per cycle) %	0.0	9.1	8.0	7.7	25.0	7.4
POMM (all cycles)						
P2+ (per cycle) %	40.0	46.3	53.8	60.6	66.2	
95% CI (P2+)	26.8–54.6	34.4–58.6	40.2–66.9	42.7–76.3	43.8–83.5	
P3 (per cycle) %	5.8	7.7	10.4	13.6	17.0	
95% CI (P3)	2.0–15.1	3.1–17.0	4.6–21.2	5.8–27.6	6.6–35.5	
Data summarized over six cycles						
Number (N) of patients with severe toxicity (naïve incidence, %)	0 (0.0)	1 (16.7)	2 (20.0)	1 (14.3)	2 (40.0)	7 (17.1)
Erlotinib + radiotherapy trial						
	Erlotinib dose (mg/m <sup>2</sup> )					Total
	75	100	125			
Number of patients	6	6	8			20
Number of cycles	26	34	36			96
Number of moderate toxicity	4	8	7			19
Number of severe toxicity	2	1	4			7
Observed moderate or severe toxicity (per cycle) %	23.1	26.5	30.6			27.1
Observed severe toxicity (per cycle) %	7.7	2.9	11.1			7.3
POMM (all cycles)						
P2+ (per cycle) %	22.7	26.6	30.8			
95% CI (P2+)	11.6–39.6	18.6–36.4	18.7–46.3			
P3 (per cycle) %	5.8	7.1	8.6			
95% CI (P3)	2.2–14.8	3.4–14.2	3.7–18.7			
Data summarized over six cycles						
Number of patients with severe toxicity (naïve incidence, %)	2 (33.3)	1 (16.7)	3 (37.5)			6 (30.0)
TITE model						
Cumulative probability of severe toxicity	30.8	33.9	37.2			
95% CI	7.5–70.9	15.8–58.3	12.2–71.5			
CACY trial <sup>b</sup>						
	Dose (doxorubicin/cyclophosphamide, mg/m <sup>2</sup> )					Total
	35/500	35/600	40/500	40/600	45/500	
Number of patients	3	3	3	8	4	21
Number of cycles	7	15	14	26	10	72
Number of moderate toxicity	3	4	11	13	2	33
Number of severe toxicity	1	6	1	7	3	18



Observed moderate or severe toxicity (per cycle) %	57.1	66.7	85.7	76.9	50.0	70.8
Observed severe toxicity (per cycle) %	14.3	40.0	7.1	26.9	30.0	25.0
Data summarized over six cycles						
Number of patients with severe toxicity (naïve incidence, %)	1 (33.3)	2 (66.7)	1 (33.3)	5 (62.5)	2 (50.0)	11 (52.4)
TITE model						
Cumulative probability of severe toxicity	42.0	72.4	49.7	78.2	57.5	
95% CI	6.6–88.1	29.2–94.3	19.0–80.6	41.4–94.8	16.4–90.3	

Bold entries correspond to the RP2D of the original trial. Confidence intervals are reported in italics.

<sup>a</sup>In the aviscumine trial, all 94 cycles from the 41 patients were included in the models; the results presented per dose level are restricted to the five higher dose levels studied, at which more than one patients had been treated.

<sup>b</sup>In CACY trial, the POMM estimated probabilities are shown in supplementary Figure S2, available at *Annals of Oncology* online.

POMM, proportional odds mixed model; TITE, Time To Event; p3, probability of severe toxicity; p2+, probability of moderate or severe toxicity; 95% CI, 95% confidence interval.

such as the continuation ratio model could be used at the cost of extra parameters to estimate. This model is also useful to test the proportionality assumption using likelihood ratio tests. In addition, a simulation study showed that this POMM approach increases the accuracy of RP2D identification, despite a certain departure from this assumption [2]. Last, the assumption that dropouts only depend on progression and severe toxicity is arguable. For instance in the erlotinib and CACY trials, two patients went off-treatment for reason other than progression or toxicity. This is however not frequent as observed on the DLT-TARGETT database where <5% of the patients went off-study for other reason than toxicity or progression [1].

In POMM analysis, the repeated outcome is the worst toxicity experienced in each cycle. It therefore mixes different types of toxicities. For example in the erlotinib + radiotherapy trial, some toxicities occurred rapidly after initiating treatment (e.g. rash) while others occurred later (e.g. paronychia), but the overall rate of toxicity remains rather constant. Indeed, as already shown in simulations, only a strong cumulative effect can be detected [2]. In the CACY study, the estimated an increased probability of toxicity was of borderline significance with an effect of OR = 1.52 per extra cycle received ( $P = 0.04$ ).

The recent experience of ceritinib accelerated approval in crizotinib-resistant nonsmall-cell lung cancer illustrates the importance to evaluate effects of treatment after cycle 1 in phase I [8]. At the RP2D (i.e. approved dose), 62% of the patients had dose reduction due to toxicity; half of the time dose was reduced after cycle 3. An analysis of this trial using POMM may have detected the impact of these moderate and late effects and may have led to redefine the RP2D.

As POMM and TITE-CRM analyses provide different types of information, one approach might be more relevant in specific clinical settings. The cumulative probability is probably the best summary of data when severe irreversible toxicities, such as cardiac or renal failure, are expected, or if toxic death occurs, which is completely unacceptable regardless of the cycle of treatment. Conversely, per-cycle probability of toxicity may be more relevant for reversible and chronic toxicity such as rash, vomiting, reversible liver adverse drug reactions.

In the future, we recommend that both approaches using all the collected data should be considered at key time-points of dose-finding trials: when interim analysis is carried out for a data safety monitoring board, to select a dose for an expansion cohort, or to complete the final analysis of the trial, in order to refine the estimates of the toxicity probabilities of the RP2D.

In conclusion, dedicated methods allow rigorous analysis of adverse drug reactions occurring during all cycles of treatment to evaluate the per-cycle probability of severe or moderate toxicity, and the cumulative probability of toxicity. These analytical strategies should not require interruption of inclusions, do not increase trial duration and may even contribute to shortening trial duration. These complementary approaches should be integrated in the analysis of phase I dose-finding trials, when patients receive several cycles of treatment.

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## disclosure

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# Cytokine profile determined by data-mining analysis set into clusters of non-small-cell lung cancer patients according to prognosis

L. Barrera<sup>1,2,†</sup>, E. Montes-Servín<sup>1,†</sup>, A. Barrera<sup>3</sup>, L. A. Ramírez-Tirado<sup>4</sup>, F. Salinas-Parra<sup>1</sup>, J. L. Bañales-Méndez<sup>1,5</sup>, M. Sandoval-Ríos<sup>6</sup> & Ó. Arrieta<sup>2,4\*</sup>

<sup>1</sup>Laboratory of Integrative Immunology, National Institute of Respiratory Diseases Ismael Cosío Villegas, Mexico City; <sup>2</sup>Postgraduate Unit, Faculty of Medicine, National Autonomous University of Mexico, Mexico City; <sup>3</sup>Computer Engineering Department, Mexico's Autonomous Technological Institute, Mexico City;

<sup>4</sup>Laboratory of Experimental Oncology and Thoracic Oncology Unit, National Institute of Cancer, Mexico City; <sup>5</sup>Laboratory of Immunology, National Institute of Cardiology, Ignacio Chávez, Mexico City; <sup>6</sup>Division of Natural Sciences, Regional Center for Teacher Development and Educational Research (CRFDIES) of Sonora, Sonora, México

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**Background:** Immunoregulatory cytokines may play a fundamental role in tumor growth and metastases. Their effects are mediated through complex regulatory networks. Human cytokine profiles could define patient subgroups and represent new potential biomarkers. The aim of this study was to associate a cytokine profile obtained through data mining with the clinical characteristics of patients with advanced non-small-cell lung cancer (NSCLC).

**Patients and methods:** We conducted a prospective study of the plasma levels of 14 immunoregulatory cytokines by ELISA and a cytometric bead array assay in 110 NSCLC patients before chemotherapy and 25 control subjects. Cytokine levels and data-mining profiles were associated with clinical, quality of life and pathological outcomes.

\*Correspondence to: Dr Óscar Arrieta, Thoracic Oncology Unit, National Institute of Cancer, San Fernando 22 Sección XVI, Tlalpan, Mexico City 14080, Mexico. Tel: +52-55-56280400; Fax: +52-55-5513151223; E-mail: ogar@unam.mx

<sup>†</sup>Both authors contributed equally to this work.